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● *Original Contribution*

COMMON CAROTID ARTERY DIAMETER, BLOOD FLOW VELOCITY AND WAVE INTENSITY RESPONSES AT REST AND DURING EXERCISE IN YOUNG HEALTHY HUMANS: A REPRODUCIBILITY STUDY

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Abstract—The aim of this study was to assess the reproducibility of non-invasive, ultrasound-derived wave intensity (WI) in humans at the common carotid artery. Common carotid artery diameter and blood velocity of 12 healthy young participants were recorded at rest and during mild cycling, to assess peak diameter, change in diameter, peak velocity, change in velocity, time derivatives, non-invasive wave speed and WI. Diameter, velocity and WI parameters were fairly reproducible. Diameter variables exhibited higher reproducibility than corresponding velocity variables (intra-class correlation coefficient [ICC] = 0.79 vs. 0.73) and lower dispersion (coefficient of variation [CV] = 5% vs. 9%). Wave speed had fair reproducibility (ICC = 0.6, CV = 16%). WI energy variables exhibited higher reproducibility than corresponding peaks (ICC = 0.78 vs. 0.74) and lower dispersion (CV = 16% vs. 18%). The majority of variables had higher ICCs and lower CVs during exercise. We conclude that non-invasive WI analysis is reliable both at rest and during exercise. (E-mail: ashraf.khir@brunel.ac.uk)

Key Words: **Ultrasound, Non-invasive, Wave intensity analysis, Wave speed, lnDU loop.**

INTRODUCTION

The temporal changes in pressure and flow generated during every cardiac cycle are inextricably linked and propagate as waves along the vascular tree. Waves contain embedded information about both their origin and the tissue through which they propagate or are reflected, thereby providing insight into the dynamic interactions among the various components of the cardiovascular system. Since antiquity, the study of the arterial pulse has played a key role in the understanding of human circulation (Karamanou et al. 2015), but recent mathematical and computational developments have opened new windows for advancing our knowledge and understanding of cardiovascular mechanics and hemodynamics. Wave propagation along the vascular tree can be studied with wave intensity analysis (WIA), a powerful tool first developed by Parker and Jones (1990), involving the decomposition of pulsatile flow into its wave components (Bleasdale et al. 2003; Hughes et al. 2008; Parker 2009; Ramsey and Sugawara 1997; Sen et al. 2014; Sugawara et al. 2009).

The common carotid artery (CCA) lends itself to non-invasive investigation because of its anatomic location (Magda et al. 2013). Several carotid WIA studies have been conducted based on non-invasive CCA blood pressure and flow velocity (U) measurements, with the former derived from either CCA diameter (D) measurements (Carbone et al. 2010; Niki et al. 2002; Rakebrandt et al. 2009) or applanation tonometry, after calibration for the derivation of blood pressure-equivalent waveforms (Curtis et al. 2007). The CCA forward waves arrive from the aorta, whereas backward reflected waves return from

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the distal microvasculature (Bleasdale et al. 2003). A forward compression wave (FCW) is created by left ventricular contraction and appears in the peripheral circulation as an early-systolic wave, whereas a mid-systolic backward compression wave (BCW) results from the distal reflection of this wave at the active vascular bed, and, in the case of measurements taken at the carotid artery, the cerebral vasculature (Manisty et al. 2009a, 2009b). The advent of WIA has facilitated the investigation of various physiologic perturbations, both in healthy patients—for example, for the study of the cardiac and/or cerebral hemodynamic effects of nicotine and caffeine (Swampillai et al. 2006), heat therapy (Hatano et al. 2002) and hypercapnia (Bleasdale et al. 2003)—and in patients for the study of pathologies such as mitral valve regurgitation (Niki et al. 1999), chronic heart failure (Curtis et al. 2007; Wen et al. 2010), hyperthyroidism (Zhang et al. 2010) and Fontan circulation (Saiki et al. 2014).

As described above, surrogate signals (computed by the analysis of distally recorded blood pressure waves) require calibration to derive a blood pressure-equivalent waveform, and this inevitably gives rise to inaccuracies (Curtis et al. 2007; Meinders and Hoeks 2004; Van Bortel et al. 2001; Zambanini et al. 2005). To circumvent this problem, recent theoretical work yielded an algorithm incorporating non-invasive *D* (instead of arterial blood pressure) and *U* measurements when determining local wave speeds and when implementing WIA (Feng and Khir 2010). This progress paralleled technological developments that led to a new generation of ultrasound machines with the capacity for direct and simultaneous non-invasive measurements of arterial *D* and *U*, through echo-tracking and Doppler ultrasound, respectively. A simple physiologic perturbation that may provide a substantial challenge to the implementation of this methodology is exercise. However, unlike other local techniques, WIA applied in the CCA may be uniquely qualified to investigate the cardiac–cerebrovascular interaction under this complex physiologic perturbation. For this endeavour to be successful, the ability to obtain reproducible CCA *D* and *U* measurements during rest and exercise is vital.

The aim of this study was to assess the reproducibility of non-invasive ultrasound measurements of CCA *D* and *U*, and of derived wave intensity parameters, obtained from young healthy participants at rest and during submaximal exercise. To the best of our knowledge, no studies exist on WIA reproducibility when derived from arterial diameter and velocity waveforms. Establishing WIA reproducibility will pave the way for more detailed exploration of the cardiac–cerebrovascular interaction and cerebral vascular resistance responses during exercise in humans.

METHODS

Study group

The study was approved by the Brunel University London Research Ethics Committee and complied with the guidelines of the Declaration of Helsinki. Twelve healthy volunteers (aged 27 ± 2 y, 6 females, body mass: 66.9 ± 5.7 kg, height: 1.69 ± 0.1 m, body mass index: 23.3 ± 1.2 kg/m^2) participated in the study after providing informed written consent. All participants were familiar with cycling, but none was a trained athlete. The participants' average daily physical activity levels were within the normal range for a sedentary to moderately active population (Sallis et al. 1985).

Instrumentation and measurements

An SSD-5500 ultrasound system (Aloka, Tokyo, Japan) equipped with a 7.5-MHz linear array vascular probe was used. The ultrasound echo tracking subsystem measured *D* with a resolution of 0.013 mm, whereas the Doppler subsystem measured *U* with a resolution of 0.012 m/s. The scans were performed in the longitudinal view, obtaining therefore images of the longitudinal section of the artery, approximately 2 cm proximal to the bifurcation. The images were optimised to ensure that the depth was as shallow as possible and the vessel walls well delineated (clear discrimination amongst the lumen, media–intima and adventitia). The gates were positioned manually in B-mode between the media and intima of the anterior and posterior walls, and parallel to them. The Doppler gate was positioned at the centre of the vessel, parallel to the walls, ensuring that the insonation angle was always between 58° and 60°. The B- and M-modes were then simultaneously displayed on a split screen, and the *D* waveform was calculated as the distance between the two walls over time obtained from the M-mode tracing. The *U* waveform was obtained from the pulsed-wave Doppler mode. Both *D* and *U* were sampled at 1000 Hz (Fig. 1a). Every measurement consisted of the simultaneous recording of *D* and *U* signals for at least 6 s. A supine bicycle ergometer, mounted on a bed and equipped with a power control box, was used to perform the exercise protocol (Angio, Lode, Groningen, Netherlands) (Fig. 1b).

Protocol

The participants were tested twice over two consecutive days and at the same time of the day. Temperature and humidity in the laboratory did not differ between the 2 d (*i.e.*, ~20°C and ~40%, respectively). Volunteers were asked to refrain from vigorous exercise and caffeine consumption for 24 h and 12 h, respectively, before the laboratory visits and to maintain the same diet on the 2 d of testing. The tests were conducted with the participants in a reclined position and their upper bodies in the

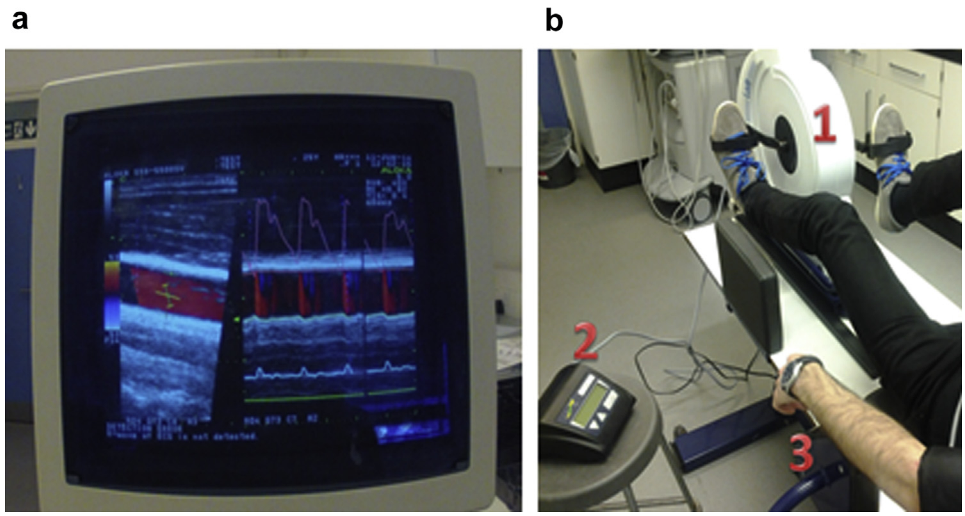

Fig. 1. Experimental setup. (a) Monitoring of ultrasound system while acquiring carotid images and Doppler information. In the frozen image on the monitor is the common carotid artery, together with the diameter waveform (*pink*) and blood velocity contour (*blue*) on the right side of the screen. (b) Semi-recumbent cycle ergometer and control box: (1) ergometer, (2) control box, (3) handle for the left arm.

semi-recumbent position (angle 32°). The participants were not restrained, but they were asked to minimize head movement and maintain a stable position by holding the bed's handles, especially during exercise (Fig. 1b).

A total of 12 measurements were taken during each day of testing, 6 at rest and 6 during exercise. Exercise measurements involved participants cycling for 2–3 min at a low cadence (30–50 rpm) and low work rate (20–40 W) (warmup period), before increasing to a cadence of 60 rpm and work rates of 80 W for males and 50 W for females (exercise period), corresponding to ≈30%–35% of the participants' maximum workload on a bicycle ergometer and consistent with the classification of participants as sedentary to moderately active (Fletcher et al. 2013; Sallis et al. 1985).

The recording of the 6 exercise measurements started 3 min into the exercise period, while the participant continued cycling. The whole protocol (warmup and exercise) lasted approximately 20 min. After completion of the measurements, the workload was decreased and the subject continued cycling for 1 min (cool-down period). Volunteers had visual feedback to maintain a constant cadence.

Data analysis

Data analysis was performed in MATLAB (Version R2010 b, The MathWorks, Natick, MA, USA). A second-degree Savitzky–Golay filter (Savitzky and Golay 1964) with a 16-point half-width window was applied to both D and U waveforms to eliminate high-frequency noise (Fig. 2). For every participant, six measurements were recorded during four conditions: rest and exercise during the first day (Rest 1 and Exercise 1, respectively) and rest and exercise during the second day (Rest 2 and Exercise 2, respectively). For each measurement, the best-quality consecutive heart cycles were selected for further analysis (at least three), based on whether they retained typical physiologic features (such as the dicrotic notch) and presented no obvious drift or dampening. For the cardiac cycles selected from each measurement, dD and dU were calculated as the incremental differences between adjacent elements of D and U, respectively.

The following features were extracted from D and U each cardiac cycle: (i) peak (systolic) diameter (D_{max}); (ii) change in diameter or pulse (ΔD), defined as the difference between peak (systolic) and trough (diastolic) values; (iii) peak (systolic) velocity (U_{max}); (iv) change in velocity or pulse (ΔU), derived similarly to ΔD (Fig. 3). The variances of dD and dU were calculated by subtracting the mean ($\bar{\cdot}$) from the instantaneous signal ($\cdot_k$), where k represents the kth element of the signal:

$$\sigma^2_{dD} = \frac{1}{N}\sum_{k=1}^{N}\left(dD_k - \overline{dD}\right)^2 \tag{1}$$

$$\sigma^2_{dU} = \frac{1}{N}\sum_{k=1}^{N}\left(dU_k - \overline{dU}\right)^2 \tag{2}$$

where N is the total number of data points included in the segment selected from each measurement. Variable σ^2_{dD} has units of mm^2, and σ^2_{dU}, units of m^2/s^2.

Assuming that reflected waves are absent during the early-systolic portion of each cardiac cycle, wave speed (m/s) for each analysed beat was calculated from the

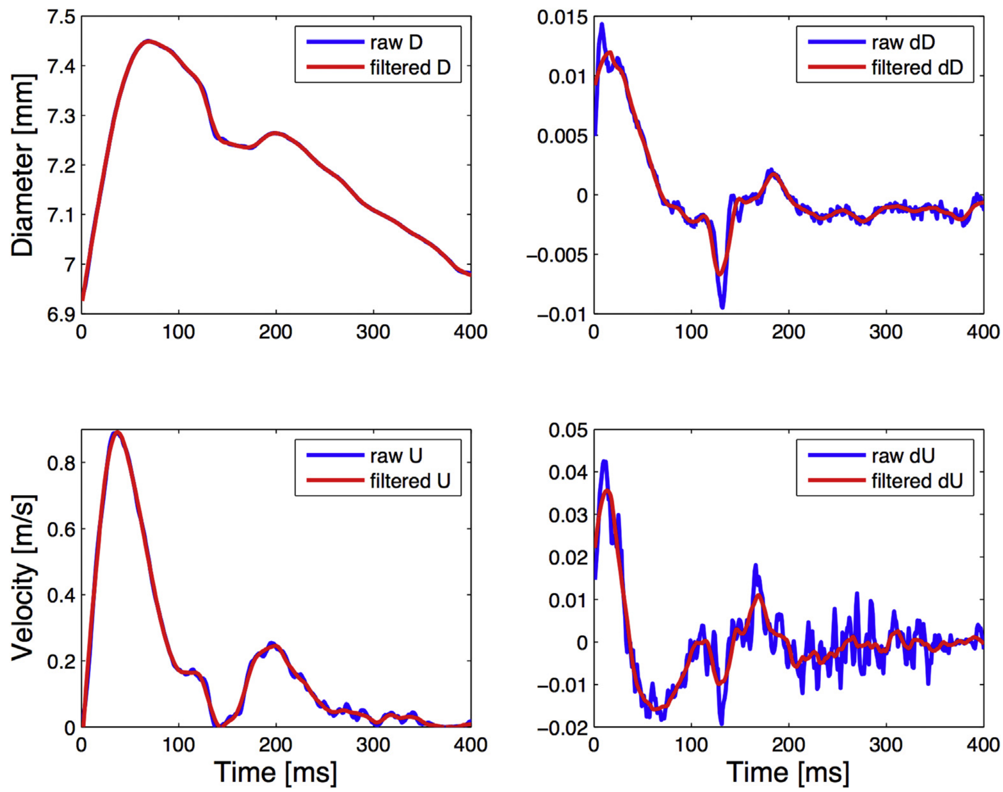

Fig. 2. Comparison between raw and Savitzky–Golay smoothed signals. Top: *D* and *dD* waveforms; Bottom: *U* and *dU* waveforms.

initial slope of the lnDU loop as (Feng and Khir 2010) (Fig. 4)

$$c = \frac{1}{2}\frac{dU}{d\ln D} \tag{3}$$

Wave intensity (m^2/s) was computed as the product $dI = dD \cdot dU$ and was separated into forward (dI_+) and backward (dI_-) components using the calculated c (Feng and Khir 2010). The peak (in m^2/s) and energy (m^2) of the forward compression wave, which is generated by the contraction of the left ventricle, were derived for each cardiac cycle from the amplitude and area, respectively, of the early-systolic peak observed in dI_+ (Fig. 3). Similarly, the peak and energy of the backward compression wave, which is attributed to reflections from the cerebral circulation, were determined for each cardiac cycle from the amplitude and area, respectively, of the mid-systolic peak that was present in dI_-. Finally, the peak and the energy of the forward expansion wave (FEW), which is generated by the deceleration of the heart's contraction, were determined for each cardiac cycle from the amplitude and area, respectively, of the late-systolic peak in dI_+. Mean values for all parameters were derived at each measurement, and further averaging yielded mean values for each day at rest and during exercise.

Statistical analysis

All values are expressed as mean ± standard deviation (SD). The statistical analyses were performed using SPSS Statistics (Version 20, IBM, Armonk, NY, USA). Tukey's test was performed for the detection of outliers (Hoaglin and Iglewicz 1987; Hoaglin et al. 1986; Tukey 1977). All variables were tested for normality using the Shapiro–Wilk test (Shapiro and Wilk 1965). Subsequently, a two-tailed Student *t*-test determined whether the data differed between testing days. The intra-class correlation coefficients (ICCs), as absolute agreement between single measures or between data set means, were calculated between the 2 d of testing, separately for rest and exercise, to provide a measure of inter-session reproducibility. The within-patient coefficient of variation (CV) between testing days was also calculated for each participant, separately for rest and exercise, and then averaged across all participants to provide a measure of the dispersion of the data around the mean during rest and exercise. Inter-observer reproducibility was assessed *via* ICCs between data set means by taking into account all measurement sessions for both testers, separating the rest and exercise data. An ICC > 0.7 was classified as "high reproducibility," an ICC < 0.5 as "low reproducibility" and an ICC between the two as "moderate

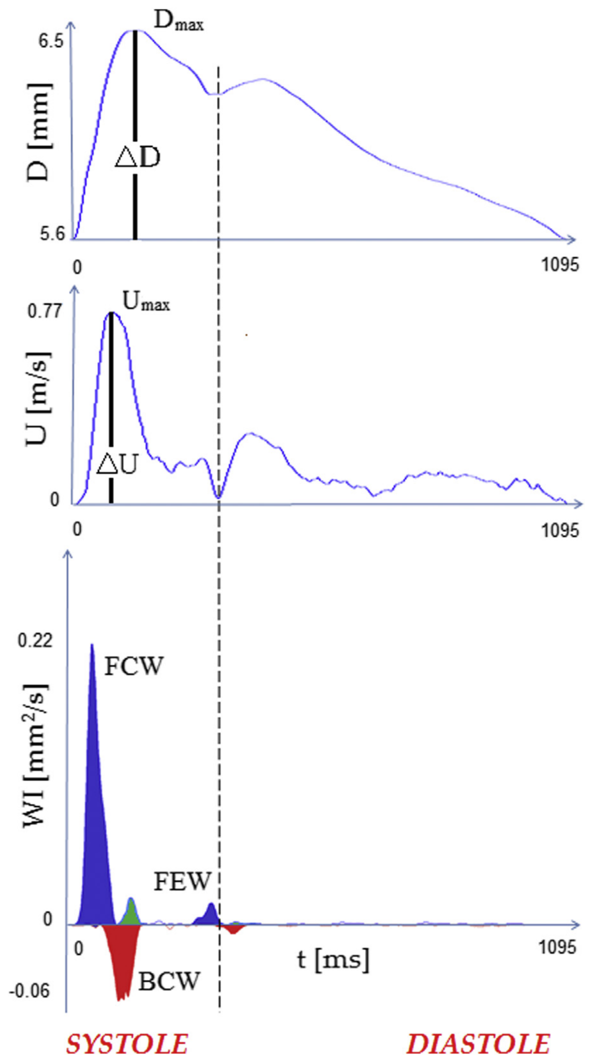

Fig. 3. Example of waveforms. Top: Carotid diameter and blood flow velocity. D_{max}, U_{max}, ΔD and ΔU are shown. Bottom: Corresponding wave intensity. The *dark blue areas* represent, from left to right, the forward compression (FCW) and forward expansion (FEW) waves; the *red areas* represent the backward compression (BCW) and backward expansion (BEW) waves, as reflections of FCW and FEW, respectively; the *green areas* represent, from left to right, the reflections of BCW and BEW. Only FCW, BCW and FEW are labeled.

reproducibility" (Portney and Watkins 2000). A CV ≥ 20% was considered "high dispersion," a CV ≤ 10% was considered "low dispersion," and the range between the two was considered "moderate dispersion." Implementing Bland–Altman analysis, the differences between two values (day 1 minus day 2) were plotted against their mean value, and the limits of agreement were defined as the mean difference ± 2SD (Bland and Altman 1986). Statistical significance was assumed for $p < 0.05$.

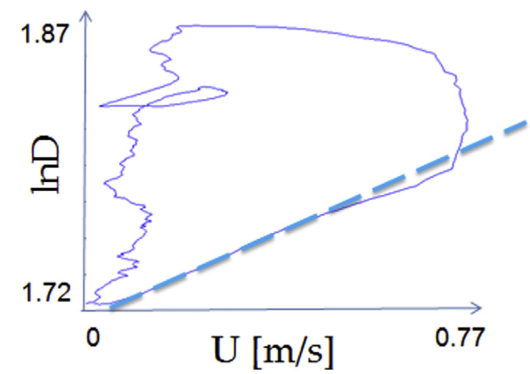

Fig. 4. Example of an lnDU loop at rest for one subject. The regression line of the early systolic linear part is shown.

RESULTS

Hemodynamic parameters

Table 1 summarises the mean values across all participants of the hemodynamic parameters under each condition. All variables were normally distributed apart from D_{max} at the first testing session (Rest 1 and Exercise 1) and σ^2_{dD} at several testing sessions and conditions (Rest 2, Exercise 1, and Exercise 2). Two outliers were observed in the same participant for the parameters D_{max} and σ^2_{dD} and were not excluded from the data sets. The *t*-tests revealed no statistically significant differences between testing days for any of the hemodynamic parameters ($p > 0.05$ for all).

The pulse values (ΔD and ΔU) had high reproducibility (greater ICC), whereas the corresponding peak values (D_{max} and U_{max}) were less reproducible, both at rest and during exercise, despite the low CV values that accompanied them. Furthermore, among these four parameters, the diameter variables exhibited generally higher reproducibility than the corresponding velocity variables at rest as well as during exercise; these findings were complemented by the CV values, which indicated higher dispersion for the velocity parameters.

In terms of derivatives, ICCs were high, supporting reproducibility of σ^2_{dD} both at rest and during exercise (Table 1). There was low (single absolute agreement) and moderate (mean absolute agreement) reproducibility of σ^2_{dU} at rest and moderate (single absolute agreement) or high (mean absolute agreement) reproducibility of σ^2_{dU} during exercise. CV magnitudes suggested lower dispersion for σ^2_{dD} compared with σ^2_{dU}.

The majority of the hemodynamic parameters had higher ICC values and lower CV values during exercise compared with rest. Only D_{max} exhibited the opposite behaviour, with lower ICC and higher CV during exercise. Figure 5a and b illustrates the individual variations within participants in mean ΔD and ΔU, respectively,

Table 1. Hemodynamic parameters at rest and during submaximal cycling*

Hemodynamic parameter	Rest 1		Rest 2	Exercise 1		Exercise 2
D_{max}						
Mean ± SD (mm)	6.91 ± 0.54		7.07 ± 0.48	7.33 ± 0.72		7.21 ± 0.49
ICC abs. agree. Si/Me		0.67/0.80			0.65/0.79	
CV (%)		3.4			3.9	
U_{max}						
Mean ± SD (m/s)	0.75 ± 0.13		0.74 ± 0.15	0.91 ± 0.19		0.90 ± 0.21
ICC abs. agree. Si/Me		0.64/0.78			0.76/0.86	
CV (%)		9.4			8.9	
ΔD						
Mean ± SD (mm)	0.59 ± 0.15		0.60 ± 0.14	0.71 ± 0.16		0.74 ± 0.16
ICC abs. agree. Si/Me		0.93/0.96			0.92/0.96	
CV (%)		6.0			5.3	
ΔU						
Mean ± SD (m/s)	0.69 ± 0.16		0.69 ± 0.17	0.90 ± 0.20		0.90 ± 0.22
ICC abs. agree. Si/Me		0.74/0.85			0.78/0.88	
CV (%)		10.4			9.2	
σ^2_{dD}						
Mean ± SD (mm^2)	0.56 ± 0.23		0.53 ± 0.27	2.03 ± 1.16		1.97 ± 1.02
ICC abs. agree. Si/Me		0.82/0.90			0.91/0.96	
CV (%)		17.1			12.0	
σ^2_{dU}						
Mean ± SD (m^2/s^2)	2.00 ± 0.85		1.74 ± 0.79	6.42 ± 2.70		6.16 ± 2.82
ICC abs. agree. Si/Me		0.39/0.56			0.60/0.75	
CV (%)		29.3			22.5	

D_{max} = peak diameter; U_{max} = peak velocity; ΔD = diameter pulse; ΔU = velocity pulse, σ^2_{dD} = variance of dD; σ^2_{dU} = variance of dU; ICC abs. agree. Si/Me = absolute agreement intra-class correlation coefficient single/mean; CV = within-patient coefficient of variation; SD = standard deviation.

* Data are from 12 participants.

across testing days and conditions. Although the values for ΔD and ΔU over the whole cohort indicate that the average of neither parameter varied substantially between testing days (Table 1), the individual data indicate that for some participants the variation observed between different days was considerable, both at rest and during exercise. The comparisons between day 1 and day 2 in Figure 6 and the Bland–Altman graphs in Figure 7 illustrate that the majority of data points for the hemodynamic parameters fall within the limits of agreement, without any obvious trend or inconsistency in variability across the graph.

Wave parameters

The mean values of the wave parameters that were investigated are provided, under each condition, in Table 2, together with the results of the statistical analysis. Wave speed values were normally distributed, except at Rest 2 and during Exercise 2. FCW peak values displayed similar behaviour and were not normally distributed at Rest 2 and Exercise 2. The outlier for FCW peak was observed in the same participant as for the hemodynamic parameters, whereas the outlier for the wave speed was observed in a different participant. They were not excluded from the data sets. All other wave parameters, BCW and FEW peaks, FCW, BCW and FEW areas, were normally distributed. No significant differences between testing days were evident for any of the wave parameters ($p > 0.05$ for all).

The ICC values for wave speed exhibited moderate (single absolute agreement) or high (mean absolute agreement) reproducibility, both at rest and during exercise. FCW and BCW peaks exhibited moderate reproducibility at rest and high reproducibility during exercise, whereas the FEW peak displayed high reproducibility in both conditions. ICC mean absolute agreement coefficients were highly reproducible for all parameters and both conditions. FCW, BCW and FEW areas exhibited high reproducibility at rest and during exercise.

Coefficient of variation values for wave speed exhibited low dispersion at rest and moderate dispersion during exercise. The FCW peak values had high dispersion at rest and moderate dispersion during exercise. The BCW peak exhibited moderate dispersion at rest and high dispersion during exercise, whereas FEW peak values had low dispersion both at rest and during exercise. FCW, BCW and FEW areas exhibited low or moderate dispersion under both conditions. Overall, wave speed, BCW peak and FEW peak dispersions increased from rest to exercise, whereas FCW peak and FCW area dispersion substantially decreased. BCW and FEW areas did not exhibit a substantial change in dispersion. The ICC values increased from the rest to exercise condition for all parameters. Figure 5c–f illustrates the changes among

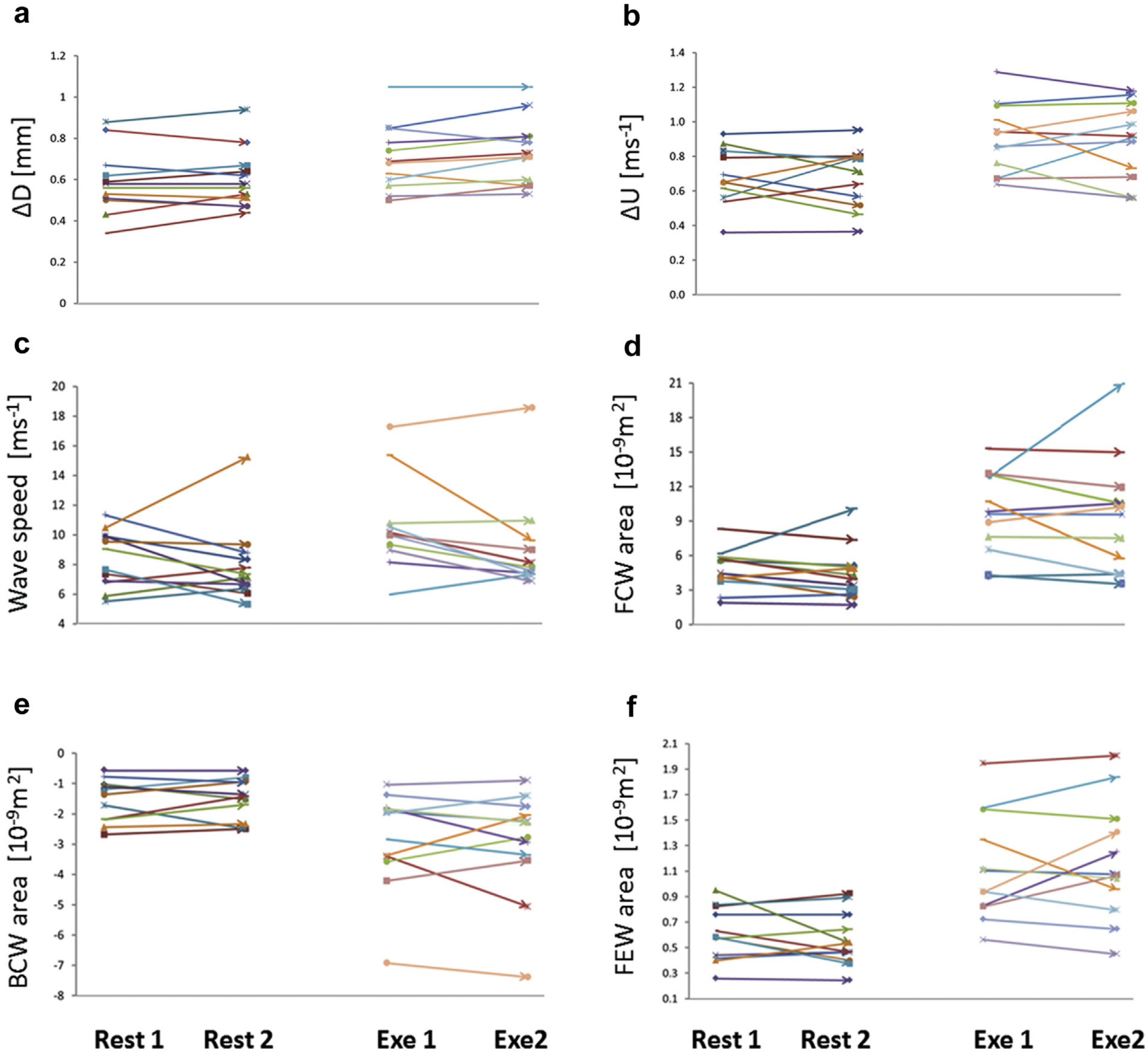

Fig. 5. Vector plots of measurements performed at rest and during exercise on both days. (a) ΔD. (b) ΔU. (c) Wave speed. (d) Forward compression wave (FCW) area. (e) Backward compression wave (BCW) area. (f) Forward expansion wave (FEW) area.

individual participants between testing days in mean wave speed, mean FCW, mean BCW and mean FEW areas, respectively, separately into rest and exercise. The averages of the whole cohort suggest neither parameter varied substantially between testing days (Table 2). The comparisons between day 1 and day 2 (Fig. 8) and the Bland–Altman graphs (Fig. 9) indicate that the majority of data points for the wave parameters fall within the limits of agreement.

Inter-observer reproducibility

Table 3 displays the mean ICC values for inter-observer reproducibility for all parameters, across all participants, separating the rest and exercise conditions. Most of the parameters exhibited a lower ICC value during exercise, although still exhibiting high reproducibility. Only the wave speed and BCW peak had moderate reproducibility during exercise.

DISCUSSION

This study illustrates that variables used to derive direct and simultaneous non-invasive wave intensity parameters are reproducible when performed at rest and during semi-recumbent cycling at moderate intensities (30%–40% of maximum workload). The non-invasive measurements of blood pressure and flow velocity to perform WIA were first suggested by Sugawara et al.

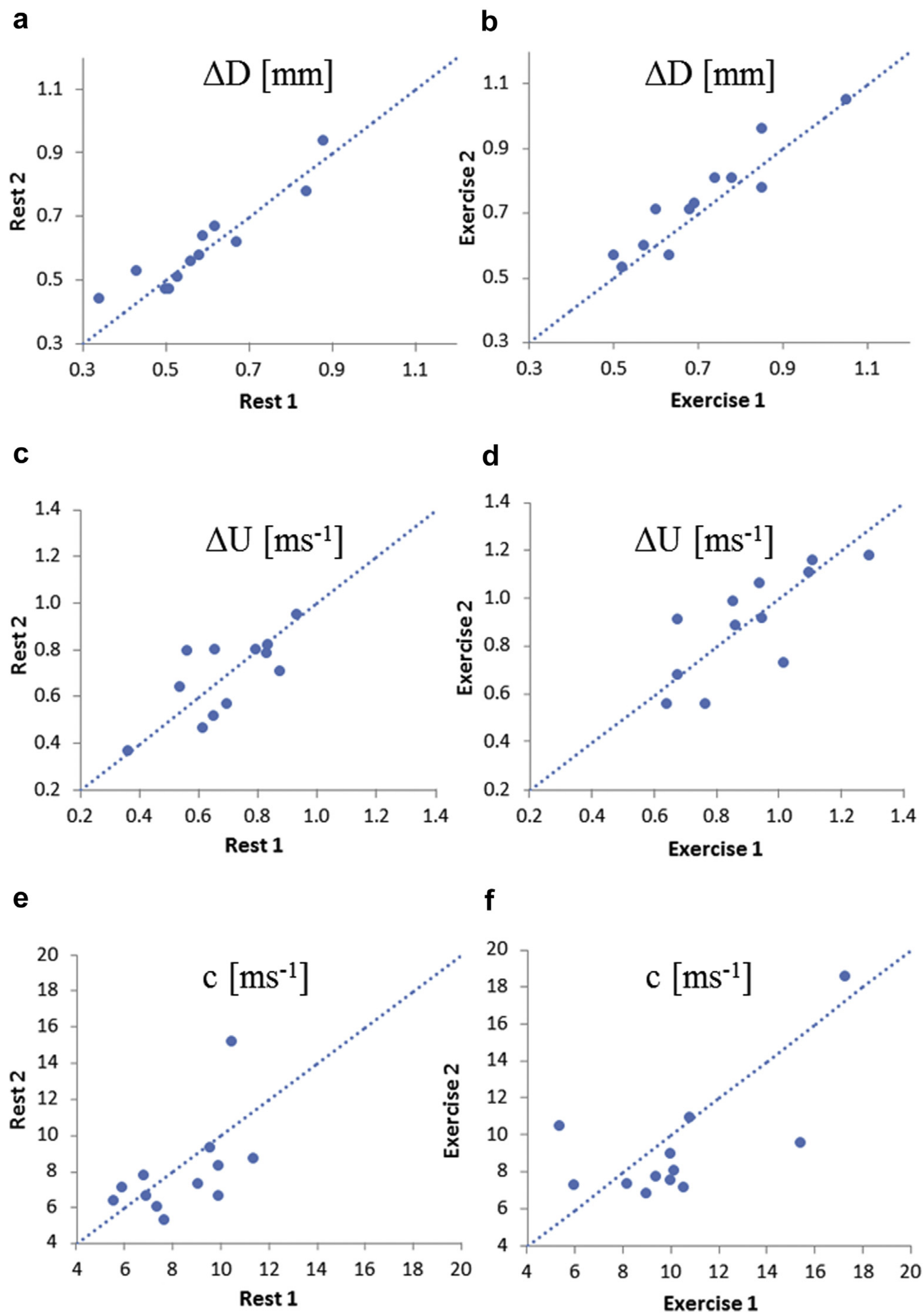

Fig. 6. Comparison of ΔD (a,b), ΔU (c,d) and wave speed (e,f) values between the 2 d of testing during rest and exercise for the whole cohort.

(2000), who found a linear relationship between pressure and diameter waveforms throughout the whole cardiac cycle. Thus, the analysis relied upon measuring diameter and velocity from the carotid artery and obtaining a surrogate of pressure waveform by scaling the diameter to brachial pressure values recorded *via* a cuff-type manometer (Niki et al. 2002). The reproducibility of this methodology was found clinically acceptable, although FCW and FEW had high variability (Niki et al. 2002). The shortcoming was that pressure contours quantitatively and qualitatively change along the arterial tree because of wave reflections (Esper and Pinsky 2014); therefore, the

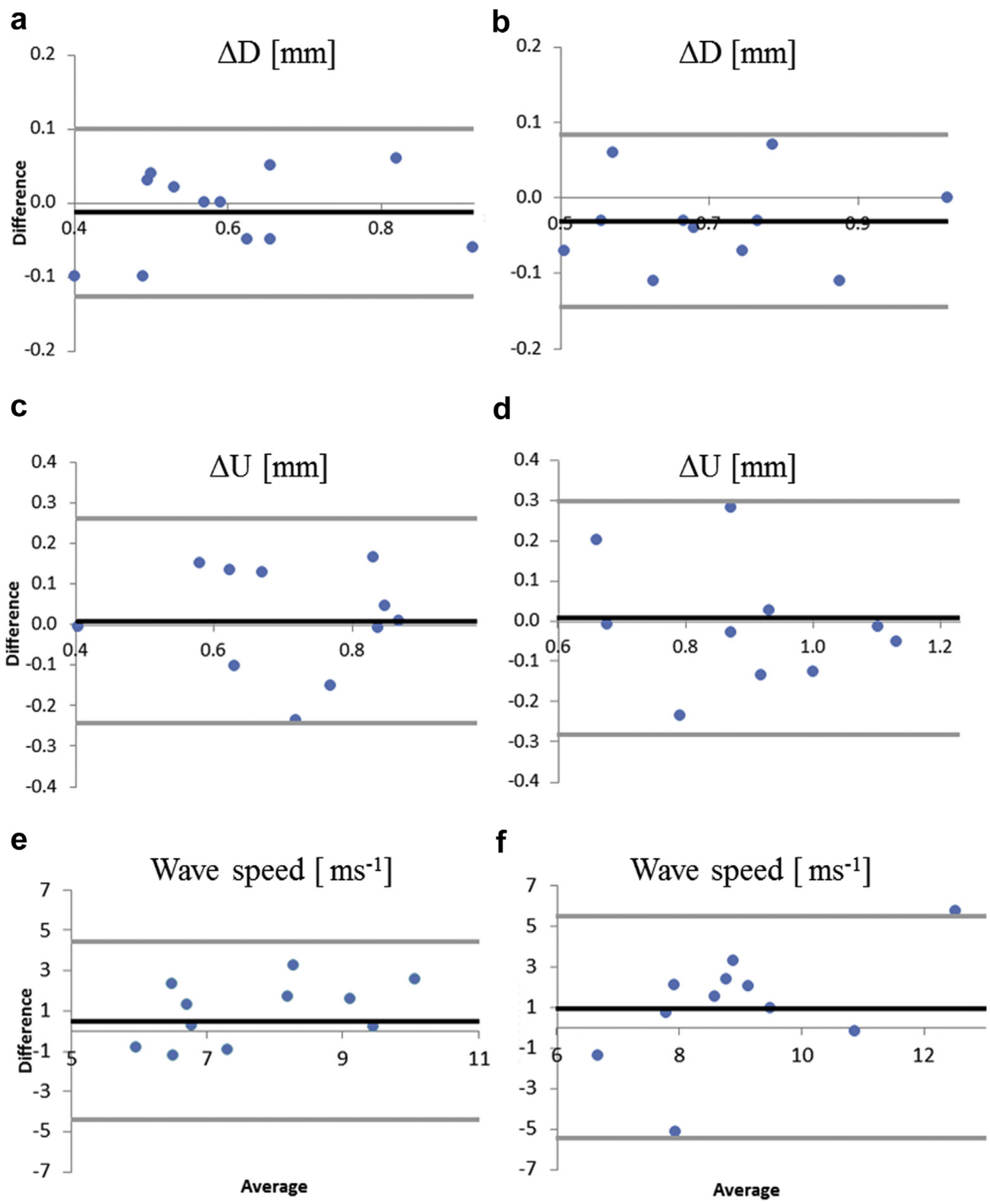

Fig. 7. Bland–Altman plots of ΔD (a,b), ΔU (c,d) and wave speed (e,f) values for the 2 d of testing during rest and exercise for the whole cohort. (a,c,e) Rest condition. (b,d,f) Exercise. The *dark thick line* represents the mean difference; the grey lines, ±2 standard deviations.

process of scaling the diameter waveform, recorded from a specific artery, to pressure data recorded from a different artery, inevitably led to errors. To overcome this problem, applanation tonometry for non-invasive measurements of blood pressure, introduced by Matthys and Verdonck (2002), was applied to WIA (Curtis et al. 2007; Zambanini et al. 2002). With this technique, simultaneous measurements of pressure and velocity at the same location are not possible, thus limiting its application when rapid physiologic perturbations, such as those introduced by exercise conditions, must be taken into account. Further development led to a direct and simultaneous non-invasive methodology, which does not need pressure recordings, but only D and U (Feng and Khir 2010). To the best of our knowledge, this methodology has only been tested *in vitro* (Li and Khir 2011), and the present study is the first to test it *in vivo* under acute physiologic perturbations.

In our analysis, pulse variables (ΔD and ΔU) were more reproducible than the corresponding peak variables (D_{max}, U_{max}), and generally, variables related to diameter measures exhibited higher reproducibility

Table 2. Wave parameters at rest and during submaximal cycling*

Wave parameter	Rest 1		Rest 2	Exercise 1		Exercise 2
c						
Mean ± SD (m/s)	8.36 ± 1.92		7.92 ± 2.58	10.15 ± 3.37		9.26 ± 3.23
ICC abs. agree. Si/Me		0.54/0.70			0.65/0.79	
CV (%)		14.6			17.1	
FCW peak						
Mean ± SD (10^{-6} m^2/s)	0.16 ± 0.06		0.15 ± 0.10	0.39 ± 0.16		0.39 ± 0.23
ICC abs. agree. Si/Me		0.65/0.79			0.72/0.84	
CV (%)		21.8			15.3	
FCW area						
Mean ± SD (10^{-9} m^2)	4.83 ± 1.76		4.50 ± 2.32	9.66 ± 3.56		9.53 ± 5.00
ICC abs. agree. Si/Me		0.73/0.84			0.76/0.86	
CV (%)		17.1			13.7	
BCW peak						
Mean ± SD (10^{-6} m^2/s)	−0.05 ± 0.02		−0.05 ± 0.02	−0.13 ± 0.08		−0.13 ± 0.07
ICC abs. agree. Si/Me		0.69/0.82			0.80/0.89	
CV (%)		18.5			22.3	
BCW area						
Mean ± SD (10^{-9} m^2)	−1.52 ± 0.70		−1.50 ± 0.65	2.85 ± 1.61		2.97 ± 1.77
ICC abs. agree. Si/Me		0.78/0.87			0.88/0.94	
CV (%)		18.6			18.4	
FEW peak						
Mean ± SD (10^{-6} m^2/s)	0.04 ± 0.01		0.03 ± 0.01	0.06 ± 0.02		0.06 ± 0.02
ICC abs. agree. Si/Me		0.77/0.87			0.81/0.89	
CV (%)		12.5			15.0	
FEW area						
Mean ± SD (10^{-9} m^2)	0.60 ± 0.21		0.56 ± 0.21	1.13 ± 0.41		1.17 ± 0.46
ICC abs. agree. Si/Me		0.70/0.82			0.84/0.91	
CV (%)		14.6			13.1	

c = wave speed; FCW = forward compression wave; BCW = backward compression wave; FEW = forward expansion wave; ICC abs. agree. Si/Me = absolute agreement intra-class correlation coefficient single/mean; CV = within-patient coefficient of variation; SD = standard deviation.
* Data are from 12 participants.

and lower dispersion than the velocity variables at rest and during exercise. The majority of the hemodynamic parameters had greater reproducibility and lower dispersion during exercise compared with rest. Overall, reproducibility increased from rest to exercise for all wave parameters. The dispersion increased from rest to exercise for the wave speed, BCW peak and FEW peak, whereas it substantially decreased for FCW peak and FCW area. BCW and FEW areas did not exhibit a substantial change in dispersion from rest to exercise. Almost all the hemodynamic and wave parameters were normally distributed.

In relation to previous studies examining dynamic parameters of the carotid arteries, our findings are consistent with those of Studinger et al. (2003), who examined arterial diameters in high resolution, and Hellstrom et al. (1996), who examined blood velocities. Previous studies have pointed out several factors that affect the reproducibility of parameters that can be used to derive WIA variables. Specifically, Peters et al. (2001) support the notion that reproducibility is generally affected most by the velocity measurement, rather than diameter. Furthermore, Deane and Markus (1997) found at the CCA that although the posterior-lateral approach offers optimal results compared with the anterior approach, both methods give reproducible data sets. We used an anterior approach to insonate the CCA for technical reasons, mainly because of the experimental setup. In addition to inherent variabilities in the signals obtained from humans, Beales et al. (2011) suggest that the main reason for poor overall reproducibility relates to operator variability. They also suggest that using the trailing edge to leading edge borders to delineate arterial diameters improves image reproducibility alongside the use of high-resolution machines (>5 MHz). In the present study these suggestions were followed to limit the impact of these issues.

Reproducibility of parameters

Diameter and velocity. The variability in D_{max} and U_{max} values may be due to superimposed respiration patterns, which can shift the waveform with the breathing cycle both at rest and during exercise. D_{max} and U_{max} are substantially affected by this type of drift, whereas the calculation of pulse tends to eliminate it. In agreement with this reasoning, ΔD and ΔU exhibit better reproducibility than D_{max} and U_{max}, both inter-session and inter-observer; nonetheless, all four parameters have good reproducibility.

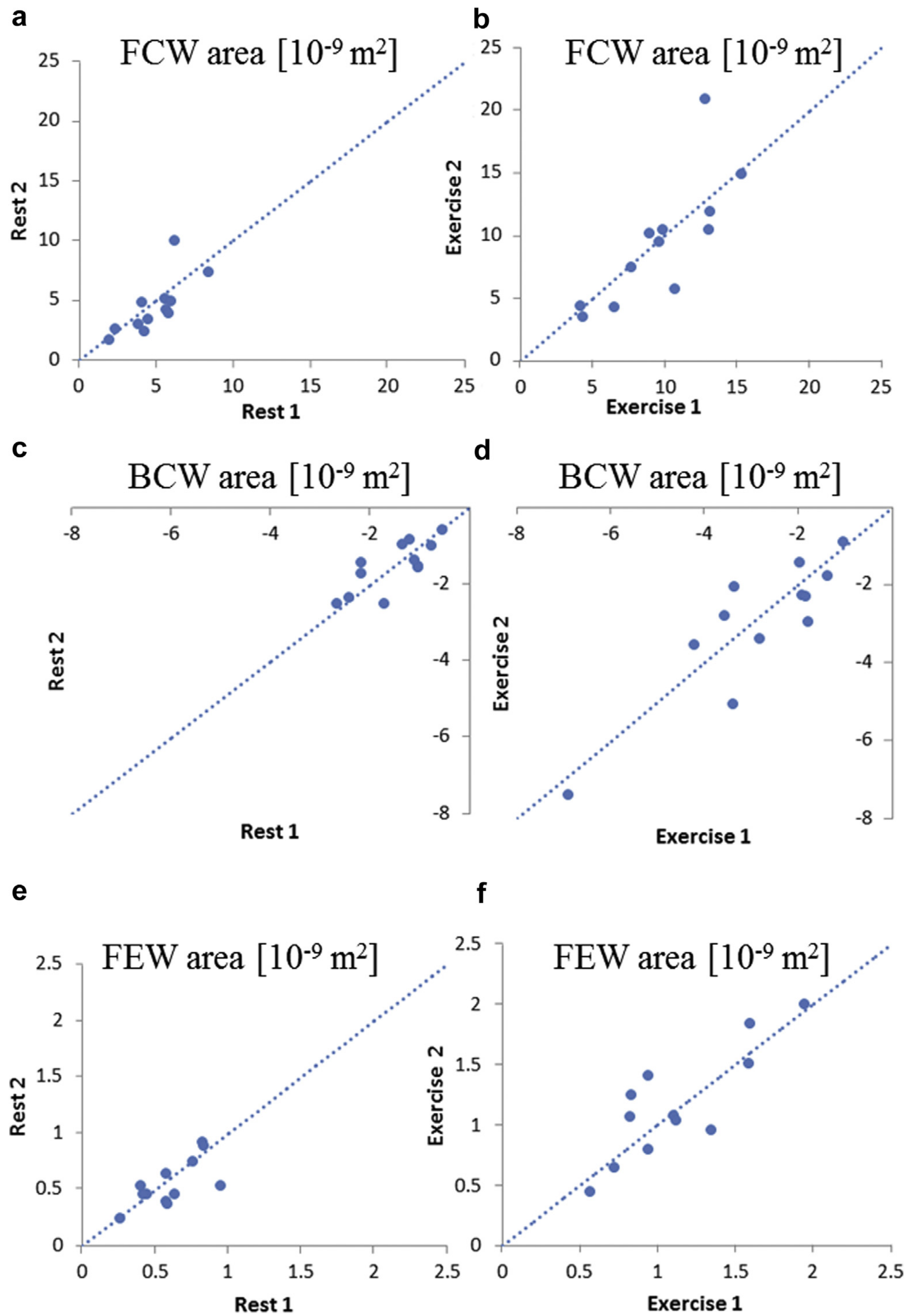

Fig. 8. Comparison of forward compression wave (FCW) (a,b), backward compression wave (BCW) (c,d) and forward expansion wave (FEW) (e,f) area values between the 2 d of testing during rest and exercise for the whole cohort.

Derivatives are limited by their computation. Factors related to the computation of the derivatives could be affecting the reproducibility of *dD* and *dU*. The filtering of the raw data preceded any other form of data processing. One of the most commonly used filters for WIA is the Savitzky–Golay filter (Savitzky and Golay 1964), but, as was recently reported (Rivolo et al. 2014), the derivatives of the smoothed signals seem to be more affected

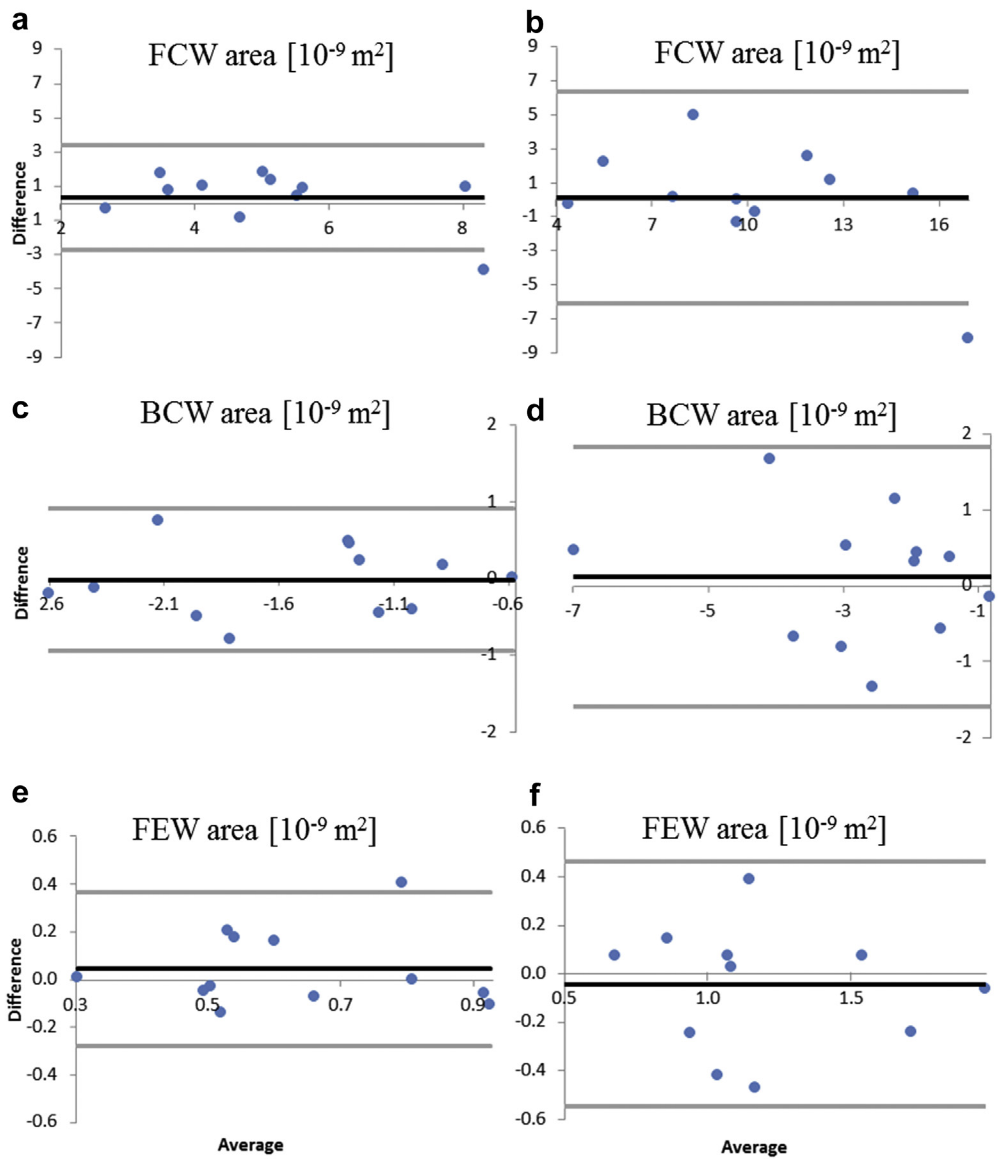

Fig. 9. Bland–Altman plots of forward compression wave (FCW) (a,b), backward compression wave (BCW) (c,d) and forward expansion wave (FEW) (e,f) area values between the 2 d of testing during rest and exercise for the whole cohort. (a,c,e) Rest condition. (b,d,f) Exercise. The *dark thick line* represents the mean difference; the grey lines, ±2 standard deviations.

by the filter parameters than the smoothed signals themselves. In addition, the noise level superimposed over any waveform increases when calculating its derivative, as well as the average error. Considering a measurement error of D and U equal to their respective resolutions (0.013 mm and 0.012 m/s) can lead to propagation errors equal to 0.026 mm and 0.024 m/s for the D and U derivatives, respectively, as well as for ΔD and ΔU (Joint Committee for Guides on Metrology 2008). The propagation errors are small compared to ΔD and ΔU values (5% and 3%, respectively) and are not expected to affect the results significantly. It could thus be anticipated that D and U would exhibit better reproducibility than dD and dU, respectively, merely because of the higher levels of noise present in the latter signals; therefore, ΔD and ΔU exhibiting better reproducibility than σ^2_{dD} and σ^2_{dU}, respectively, is not unexpected.

For the assessment of dD and dU reproducibility we used comprehensive parameters which are calculated using every single element of the signals, whereas for the assessment of D and U reproducibility, only two elements (peak and trough) of the corresponding waveforms were used. Even though the latter method is inherently more vulnerable to noise and artefact interference, it appears that the added noise present in the derivative signals results in σ^2_{dD} and σ^2_{dU} being less reproducible than ΔD and ΔU.

Table 3. Assessment of inter-observer reproducibility for the hemodynamic and wave parameters, at rest and during submaximal cycling*

Parameter	ICC rest	ICC exercise
D_{max}	0.76	0.81
U_{max}	0.94	0.72
ΔD	0.94	0.95
ΔU	0.97	0.72
σ^2_{dD}	0.91	0.91
σ^2_{dU}	0.85	0.81
c	0.84	0.56
FCW peak	0.96	0.90
FCW area	0.94	0.83
BCW peak	0.77	0.67
BCW area	0.87	0.71
FEW peak	0.72	0.79
FEW area	0.88	0.73

D_{max} = peak diameter; U_{max} = peak velocity; ΔD = diameter pulse; ΔU = velocity pulse; σ^2_{dD} = variance of dD; σ^2_{dU} = variance of dU; c = wave speed; FCW = forward compression wave; BCW = backward compression wave; FEW = forward expansion wave; ICC = intra-class correlation coefficient.

* Data are from 12 participants.

Although D and U are measured simultaneously and by the same probe, the manual adjustments of gates and angles may affect each signal differently, and this may be more evident among derivatives, especially dU. Regardless, there were no substantial differences, neither for σ^2_{dD} nor for σ^2_{dU}, between the 2 d of testing, neither at rest nor during exercise, suggesting that with an adequate sample size this inconsistency may be overcome.

Wave speed can be determined at rest and during exercise non-invasively. Analysis of wave speed ICC values revealed moderate-to-high reproducibility, both inter-session and inter-observer. The calculation of wave speed is intimately connected to the ratio of pulse variables (ΔU and ΔD), as highlighted by eqn (3), and it is mainly affected by the reproducibility of velocity variables. The inaccuracies in measuring velocity are inherent to the methodology itself. In fact, the actual ultrasound measurement of velocity is a combined signal of all the red blood cells that are causing the Doppler frequency shift. This is not uniform from beat to beat, because the blood flow and the red blood cells entering the control volume (region of interest) and making up the shift inherently change from beat to beat, whereas the diameter is determined by tracking the same control volume throughout. What the ultrasound probe records is only an average over the cross-sectional area of the vessel. Therefore, the wave speed reproducibility cannot be higher than or similar to the reproducibility of diameter variables. Nevertheless, the lnDU loop technique may have played (and may play, in general) an important role in affecting the reproducibility, as addressed under Experimental Considerations and Limitations, because of the linearity threshold settings.

Wave intensity. Energy (area) reproducibility is more consistent than peak energy parameters, both inter-session and inter-observer. This is probably due to the area calculation taking into account a whole segment of the waveform, rather than just a single point. The latter is more susceptible to noise and artefact interference than the former. Nevertheless, both areas and peaks exhibited a generally high degree of reproducibility.

Implications for future work

Despite a number of potential error sources, we have explained that we can obtain D and U measurements and perform direct and simultaneous non-invasive WIA reliably both at rest and during exercise. Unlike other local techniques, such as near-infrared spectroscopy, non-invasive WIA is uniquely qualified to investigate the coupling of the heart with the cerebral vasculature. Furthermore, contrary to magnetic resonance imaging-based techniques, non-invasive WIA can provide results at sub-beat temporal resolution, using lower cost equipment.

Experimental considerations and limitations

Data from two participants (one male) produced outliers in some, but not all, data sets (D_{max}, σ^2_{dD}, FCW peak, c). Further investigation did not highlight any qualitatively unphysiologic waveform or error in the analysis. Therefore, we believe the corresponding data points represent real variation and cannot be dismissed. Although the ensemble averaging process is more common in the analysis of ultrasound measurements, we judged that it could lead to miscalculations of wave speed values, which are strictly dependent on the early upstroke of the waveforms; consequently, erroneous estimates of WI parameters could be created in our study. Therefore, we chose to calculate all the parameters from individual cardiac cycles and then average the results. This method may have contributed to lower theoretical reproducibility values and must be considered in relation to previous work. On the other hand, the wave speed derived from the lnDU loop has been derived visually from the optimal linear fit over the early systolic part of the loop. Although repeating the calculations manually gave very similar results, the possibility exists that the use of an automatic linear regression algorithm would reduce the inter-observer variability. However, a drawback associated with the use of an automatic linear regression algorithm could be the assessment of a proper "linearity threshold," which may in turn be data set dependent (Khir et al. 2007). Finally, the lack of electrocardiogram and breath frequency monitoring to assess the impact of heart rate and breathing frequency on the variability of the data in the present study is a limitation that warrants further investigation in future studies.

CONCLUSIONS

Arterial diameter and blood flow velocity waveforms, obtained from ultrasound measurements of the CCA of young healthy participants, are fairly reproducible, together with all derived parameters, including wave intensity, both at rest and during submaximal semi-recumbent exercise. This outcome opens up new possibilities for non-invasive investigation of cardiovascular properties under physiologic perturbations.

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